IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Toshiyuki MIYATA et al.

Serial No.: 10/531,427 Group Art Unit: 1656

Filed: April 15, 2005 Examiner: Alexander D. KIM

For: SUBSTRATE POLYPEPTIDES FOR VON WILLEBRAND FACTOR CLEAVING PROTEASE ADAMTS-13

DECLARATION UNDER RULE 1.132

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Sir:

I, Koichi KOKAME, citizen of Japan declare and say that:

1. I was graduated from Department of Biophysics, Faculty of Science, Kyoto University, Japan in March, 1989.

2. I was graduated from Master Course, Department of Biophysics, Faculty of Science, Kyoto University, Japan in March, 1991.

3. I was graduated from Doctor Course, Department of Biophysics, Faculty of Science, Kyoto University, Japan in March, 1994.

4. I was awarded the degree of Ph.D. of Science in March, 1994. From April, 1994 to August, 1994, I was a Research Fellow of Japan Society for the Promotion of Science, Department of Life Sciences, Graduate School of Arts and Science, University of Tokyo, Tokyo, Japan. From September, 1994 to March, 2002, I was a staff of National Cardiovascular Center Research Institute, Osaka, Japan. From April, 2002 up to this time, I have been

a Laboratory Chief, National Cardiovascular Crnter Research Institute, Osaka, Japan.

5. I am one of the inventors of the above-identified application and am familiar with the subject matter thereof.

6. I have read the Office Action mailed October 19, 2006 and am familiar with the subject matter thereof.

7. In order to show that a skilled person in the art can easily practice the inventions relating to the mutant polypeptides as claimed, the following experiments have been done under my direction.

(1) Materials and Methods

(a) Test polypeptides

The mouse polypeptide was a polypeptide GST-Asp1596-Arg1668-H derived from mouse VWF.

The human polypeptide was a polypeptide GST-Asp1596-Tyr1668-H derived from human VWF (i.e. GST plus SEQ ID NO: 5).

As shown in the Figure below, the mouse polypeptide has an identity of 86.3% (63/87 residues)(i.e. <90% identity).

(b) Preparation of test peptides

The mouse polypeptide was prepared according to the procedure described below, which is substantially same procedure described in the working examples of the specification.
To construct a expression vector for partial regions of mouse

VWF tagged with glutathione S-transferase (GST) and 6xHis (H), the Asp1596-Tyr1668 region of VWF was amplified by polymerase chain reaction (PCR) using total RNA prepared from mouse livers. The PCR product was ligated into the expression vector.

To obtain the recombinant polypeptide, the GST-Asp1596-Tyr1668-H expression vector was introduced into E coli. The bacterial cells were collected, lysed, and centrifuged to collect soluble fractions.

GST-Asp1596-Tyr1668-H in the fractions was purified by Ni-resins and GST-resins.

The human polypeptide was prepared according to the same method described in the working examples in the specification.

(c) Reaction with human plasma

The mouse polypeptide was reacted with human plasma according to the procedure described below, which is substantially same procedure as described in working example in the specification. Purified GST-Asp1596-Tyr1668-H was incubated with human plasma at 37°C. The samples were subjected to SDS-PAGE and Western blotting using anti-GST antibodies.

Purified human polypeptide was reacted according to the same method described in the working examples in the specification.

(d) Results

As shown in Figure below, both the mouse polypeptide and the human polypeptide were cleaved by human plasma, and the cleaved product was clearly detected.

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Human  DREQAPNLVY MVTGNPASDE IKRLPGDIQV VPIGVGPNAN VQELERIGWP NAPILIQDFE TLPREAPDLV LQR
       **  ****** ********** ********** ******* **  ******  *  *** * *** ********** **
Mouse  DRVEAPNLVY MVTGNPASDE IKRLPGDIQV VPIGVGPHAN MQELERISRP IAPIFIRDFE TLPREAPDLV LQT
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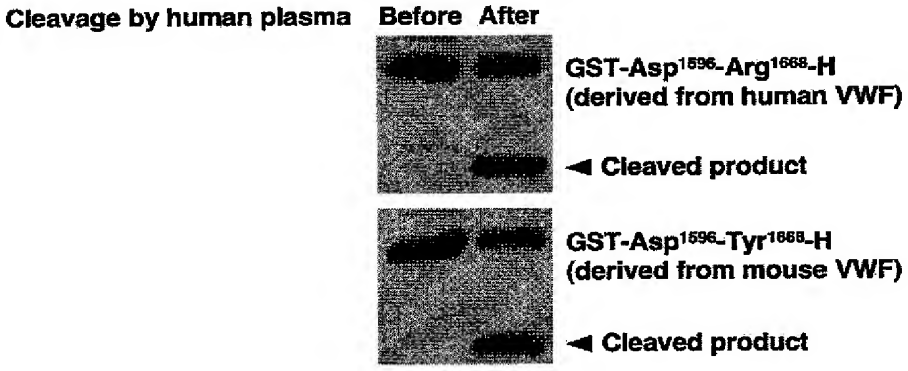
86.3% Identity (63/73 residues)



6. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 29th day of November , 2007

Koichi KOKAME